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Dockets Management Branch (HFA - 305)
5630 Fishers Lane, rm. 1061
Rockville, MD 20852.
RE: Docket #'s 99D-4488, 99D-4489

12/8/99

To whom it may concern:

This letter is in response to the recently published FDA Guidance documents related to the production of sprouts. It is intended to complement an earlier response letter dated 11/8/99, which was submitted prior to the 'Sprout Summit' meeting on November 15 & 16, 1999.

This letter will propose that, based on currently available evidence, a seed sampling and testing protocol, **specifically** designed for sprouting seed, could be as effective in reducing the occurrence of sprout-related disease as any presently known chemical intervention. For this reason, until other interventions can be shown to be more reliable than **pre-production** seed sampling and testing, I ask that such testing, in conjunction with production testing as outlined in the FDA Guidance document, be acknowledged by the FDA as an alternative to chemical treatments of sprouting seed.

It is my feeling that the available information on the effectiveness of chemical and irradiation treatments, as well as repeated generalizations concerning the unreliability of seed testing, which have been made in the NACMCF "White Paper" and elsewhere, are based on research which involved poor or nonexistent controls, and that for this reason, pre-production seed testing has been overlooked as a significant risk-reduction step.

For example, in the section on "Sprout-Associated Outbreaks" in the NACMCF "Microbiological Safety Evaluations and Recommendations on Sprouted Seeds," it is mentioned that researchers have been unable to detect pathogens in several seed lots which were implicated in outbreaks of sprout-related illnesses.

It is now generally recognized that detecting pathogens in sprouting seed requires

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certain sampling and testing **protocols**, which have only **recently** become **well-**understood. For this reason it is misleading to state that pathogens were not found in a seed sample, without specifying the protocols used.

There are several statements in the "White Paper," which cast doubt on the reliability of seed testing. In describing a Salmonella Stanley outbreak of 1995, "Analysis of alfalfa seeds and sprouts from the small amount of the seed lot remaining did not yield S. Stanley." Relative to a Salmonella outbreak of 1995-96, it is written "Seed samples, however, did not yield either **serotype**." Regarding a 1998 outbreak of Salmonella Senftenberg, "analysis of the clover and alfalfa seeds did not yield the pathogen." Relative to a 1998 E. coli **0157-H7** outbreak in Nevada and California, "Laboratory analysis of seeds, sprouted seeds, and environmental samples did not yield E. coli **0157:NM**."

Mentioning the inability of researchers to find pathogens by testing contaminated seeds casts doubt on the reliability of sampling and testing as a risk-reduction procedure. However, to offer such statements without specifying the sampling and testing methods used is analogous to discrediting chlorination or any other intervention without giving any specific data on concentrations or other parameters. It gives the impression that a scientific inquiry has taken place, when in fact the testing may have not been appropriate for accurate detection. In the process, it tends to discredit an approach to risk reduction which may be extremely effective

In all cases where specific sampling and testing protocols are mentioned relative to naturally or artificially contaminated seed, there is strong evidence that seed sampling, using appropriate sample sizes and testing methods, would have averted past outbreaks of illness, and could reasonably be expected to avert future ones.

In possibly the only published peer-reviewed research paper describing a specific approach to sprouting seed sampling and testing in **naturally** contaminated seed (Inami and Moter, Journal of Food Protection, June, 1999) the sample size, number of samples, method of pre-testing incubation, testing protocols, and results are all specified. The method used was able to detect pathogens using a **fairly** small total sample, and would certainly have been adequate to keep this seed lot out of production, had a similar sampling and testing protocol been used before the seed was grown for sprouting.

Even in this instance, where relatively good methods for sampling were used and documented, the incubation step as described possibly diminished the effectiveness of the procedure, since the researchers did not water the samples during four days of growth, following the initial soak. It might be that the dryness of the samples inhibited not only sprout growth, but also microbial growth prior to the actual testing, and that if the sprouts had been watered on a schedule similar to what is common in commercial sprouting, the pathogen level would have been higher, and even easier to detect.

The summary of the "Sprout-Associated Outbreaks" section of the NACMCF paper states: "Frequent failures to isolate pathogens from implicated seeds suggests that seed contamination may be intermittent, at very low levels, or unequally distributed with seed lots."

Before dismissing the whole idea of seed testing as a reliable risk-reduction measure, each of these difficulties **should** be carefully addressed, particularly in light of the risks, problems and uncertainties of any presently-known alternatives.

The "intermittent" and "unequally distributed,, problems **could** be addressed in one of two ways: either sample entire sprouting- seed lots using a method of continuous sampling during the bagging of seed, similar to that described in my initial letter, or mix any seed lot intended for sprouting, using a method which assures a high degree of uniformity, before taking the sample.

Regarding the problem of "very low levels" of contamination, available data on sampling and testing of implicated seed needs to be reviewed. On any of this seed which is still available, a random sample of 3 or more kilos needs to be taken and screened using appropriate testing, before concluding that seed sampling is not a reliable risk-reduction method. The age of the seed and probable survival rates of the pathogens in question need to be factored in to the evaluation. If no seed is available from a particular past outbreak, then the lowest observed level in available contaminated seed could be used as a basis for setting a recommended sampling amount, adding in an appropriate margin for safety.

Seed sampling and testing protocols are crucially important in the evaluation of the effectiveness of sanitation interventions, particularly relative to naturally contaminated seed. However, there is evidence that such protocols have not been used, or even proposed, relative to the FDA- recommended interventions in the "White Paper," and relative to new interventions which are being considered.

For example, in a presentation by Dr. Fett at the 11/15-16/99 "Sprout Summit" , which dealt with the effectiveness of strong **chlorine** seed-soaks, Dr. Fett described taking twelve 100 gram samples from a lot of naturally contaminated seed, using 6 of these samples for a control, and chlorinating the other 6. In testing the control group for pathogens, three gave positives, and three gave negatives. On the basis of this result, one could assume that about 50% of any number of 100 gram samples taken from this lot would produce negatives.

The 6 chlorinated samples in Dr. Fett's experiment resulted in 6 negatives. **Although** the implication of these results is that the chlorination "works," a statistical analysis of these results would be quite inconclusive, since 3 out of the 6 could be expected to be negative with no treatment.

Also, it is misleading to present kill-rates which are based on ideal lab protocols, - such as the use of buffered or purified water for chlorine solutions- rather than actual production practices, as evidence for the effectiveness of treatments, since sprouters would be using municipal- or well-water which would have quite different properties, and might therefore give different results.

In another presentation, Dr. Don Thayer described the effectiveness of gamma irradiation in reducing pathogens on sprouts. Dr. Thayer reported getting 18 consecutive positive 25 gram samples in a control group from the same contaminated seed which had given Dr. Fett 3 out of 6 positives, using 100 gram samples.

Based on Dr. Fett's control sample: 3 out of 6 positives from 100 gram samples, the contamination level of the seed could be estimated at less than **10 CFU/kg**. Using the sampling formula $P = 1 - (C/T)^N$, where P is **probability** of detection, C/T is the ratio of clean seeds to total seeds, and N is the number of seeds sampled, the likelihood of getting 18 consecutive positive 25 gram samples from this same seed is about 1.6^{-12} , or about one in a trillion.

The differences in contamination levels in the samples taken by Dr. Fett and Dr. Thayer are statistically so unlikely as to be unbelievable. To dismiss this difference as proof of the inconsistency of pathogen distribution in naturally contaminated seed is unjustified without further testing of the seed involved.

Although there was apparently some recovery of pathogens upon enrichment following the irradiation process, a chart shown during Dr. Thayer's presentation showed 18 positive samples and 18 negative irradiation results. But based on Dr. Fett's sampling, about fifteen of the eighteen 25 gram samples in the test would have been negative to begin with.

Although there is a possibility of "hot spots" in seed which may account for the differences in Dr. Fett's and Dr. Thayer's samples, the "hot spot" hypothesis cannot be assumed without also assuming the possibility of "cold spots." Without a sampling and testing protocol, negatives which are observed following interventions on naturally contaminated seed cannot be interpreted as being a result of the treatment; they may instead represent the properties of the seed before the treatment was carried out.

Since all **chemical** or irradiation tests on naturally contaminated seed appear to have been done without establishing **valid** controls, no conclusions can be drawn about the effectiveness of these interventions. Such conclusions must be borrowed from tests done with artificially contaminated seed.

However it is speculation to say that intervention results using artificially contaminated

seed can be extrapolated to naturally contaminated seed . Although it is true that in observed instances, natural contamination levels appear to be much lower than those achieved with lab inoculations, whether this contamination is more or less “superficial” one **can** only guess. Furthermore, it is generally acknowledged that microbes, including some pathogens, enter into self-protective states under different environmental conditions, such as desiccation. This characteristic might tend to make naturally occurring microbes much more resistant to a short-term chemical soak than artificially inoculated microbes from fresh lab cultures would be.

Pre-production sampling and testing would allow for much more accurate detection of these possibly dormant organisms, as well as pathogens hidden in cracks in the seed coat, or even in the tissue of the sprout, since testing could be done after a much longer period of hydration, without pressure from production schedules.

A pre-production seed sampling and testing protocol is also needed in light of the recommendations for product testing as described in the recent “Guidelines.”

The **occurrence** of presumptive positives at rates anywhere close to the 1%-2% which are common in rapid detection Salmonella kits would make it impossible to run an effective business and to supply markets at the required level of consistency.

A partial list of the consequences would include the following:

- Each presumptive will cause a logistical and ethical crisis in managing the area giving the presumptive, relative to concurrent crops in adjacent areas of the **facility**.
- There will be a crisis in customer confidence when part of a shipment is withheld because of questions about its safety, even where no safety problem exists.
- The grower will have to permanently remove any seed which gives a presumptive positive from his facility, even if the presumptive turns out to be false, since that seed can be expected to give presumptives on subsequent crops, and each presumptive will require a quarantine..
- Presumptives on any crop from a seed lot which has given negatives in prior crops will put the grower in an ethical dilemma regarding notification of customers who have already received product from this seed lot.
- In the absence of very rigorous pre-production seed sampling and testing, the grower will have no assurance that any seed he purchases will be suitable for production, even if he grows out a sample of the seed beforehand.

A pre-production seed sampling and testing protocol designed specifically for sprouting seed could eliminate these problems.

If for any reason a grower needed to continue to use a seed lot which in a **pre-**production seed testing program gives presumptive positives, but which subsequently provides a confirmed negative, -for example, a seed which could not be easily replaced- an alternative testing plan could allow the grower to stay in production. In this case, the **48-hour** rapid-detection test could be omitted, but the same sample **could** be sent to a lab equipped to do PCR testing. Although PCR is not yet AOAC approved, a negative **PCR** result, in combination with a negative from the **pre-**production testing, would add a huge margin of safety, and would allow for the use of otherwise wholesome seed which gave presumptive positives.

The FDA and CDC research indicates that all the problems associated with sprouts in the last several years, with the possible exception of a single one, have been caused by pathogens entering the sprouting facility on the seed.

It is also acknowledged that growing contaminated seed in a facility with the most sophisticated equipment, and flawless **GMP's**, **will** not eliminate the likelihood of shipping contaminated product, if the seed is contaminated.

It is recognized by the FDA that there is no presently known, reliable way to disinfect contaminated seed. Conversely, there is no evidence that sprout-growers, using reasonable care and common sense, and their own ingenuity, cannot produce safe sprouts if the seed they are using does not contain pathogens.

So, empirically, the problem could be said to be the elimination of contaminated seed from sprout-growing facilities. So far, almost all of the research inquiry into sprout safety has been based on the assumption that preventing contaminated seed from entering the sprouting facility in the first place is either impossible, or impractical.

Lately, several seed suppliers have introduced methods of seed sampling and testing for their sprouting seed. However, traditional sampling and testing methods for other types of seed are not adequate for significant risk-reduction on sprouting seed. A protocol for sprouting seed needs to be established.

The level of pathogens found in naturally contaminated seed which has been obtained by researchers is estimated (in the NACMCF White Paper) to be as low as 4 CFU per kilo. The White Paper seems to conclude that such low levels preclude effective sampling. However, sampling and testing of these observed lots of seed prior to their use could have averted the outbreaks which they caused.

A contamination **level** of 4 CFU per kilo, even if on a single seed in each kilo, could be detected to a 99.99 % probability by taking a **10** kilo random sample from the seed lot in question, growing it out, and testing the runoff from the resulting sprouts.

Although taking a 10 kilo random sample, and then growing the sample and testing the resulting sprouts, may seem impractical, it is not nearly so difficult, expensive, or dangerous as the presently recommended chlorine treatment. and it would avoid the possible pitfalls of the testing as recommended in the guidelines.

Such a sampling method would pick up "hot spot" areas in a lot of seed. A ten kilo sample taken from 10,000 pounds of seed which was either thoroughly blended, or continuously sampled in a bagging operation, would sample the equivalent of five hundred random locations in each pound of seed, or about one location per cc. The amount of seed used in a ten kilo sampling of a 10,000 lb seed lot would be 2/10 of 1%.

Such samples could be subjected to multiple testing, or testing for pathogens in addition to 0157 and Salmonella, without adding significantly to the cost of the seed, since large volumes would be involved. The integrity of the bags between sampling and end use could be assured by using contamination-resistant packaging and black-light inspection of the bags prior to use at the sprouting facility.

Thorough pre-production seed sampling and testing could preserve the sprouting industry as a unique community of inventive entrepreneurs, and would be entirely compatible with organic production requirements. It would avoid the many potential problems associated with chemical treatments, such as disposal problems, worker health problems, as well as the possibility of selecting for chlorine- or other resistance, and the likelihood of increased susceptibility to contamination of crops through the drastic reduction of background flora.

Jonathan Sprouts, for itself and on behalf of the sprout industry, asks that the FDA give this proposal its serious consideration as an alternative and complement to the recently published guidelines.

Yours truly,

A handwritten signature in black ink that reads "Bob Sanderson". The signature is written in a cursive, flowing style.

Bob Sanderson

President

Jonathan Sprouts, Inc.